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ORIGINAL ARTICLE

Bone mineral density measurements performed by cone-beam computed tomography in the bisphosphonate-related osteonecrosis-affected jaw

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Abstract
Objectives The aims of this study were to determine the bone mineral density (BMD) in bisphosphonate-related osteonecrosis of the jaw (BRONJ) by cone-beam computed tomography (CBCT) measurements and to correlate these measurements with the current stages recommended by the American Association of Oral and Maxillofacial Surgeons (AAOMS).
Methods Bone mineral density measurements of various areas in 24 bisphosphonate-related osteonecrosis (BRON) jaws were evaluated by CBCT. Another 24 age- and sex-matched patients without any bone pathologies served as the control group. Data acquisition was highly standardized to ensure maximum reliability in the comparisons of BMD measurements by CBCT.
Results Compared with the control group, the bisphosphonate patients had significantly higher ($p \leq 0.01$) BMDs in the non-affected jaw areas ipsilateral and contralateral to the BRON within the maxilla and mandible. The highest BMDs within the BRON jaws were observed in the BRON-adjacent areas relative to the non-affected ipsilateral and contralateral areas. Regarding the correlation with the AAOMS stages, the BMDs of the evaluated areas of BRONJ showed no significant differences ($p \geq 0.05$) between the stages.
Conclusions Bisphosphonate-related bone pathologies can be detected by CBCT and are associated with increased BMDs, not only in clinically obvious BRONJ areas, but also in clinically unapparent areas, suggesting a subclinical general osteosclerosis of the jaw. The data transferability to other CBCT devices needs to be further elucidated and compared with multislice CT.

Keywords Bisphosphonate · Cone-beam computed tomography · Density · Osteonecrosis · Bone mineral density

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Introduction

Over the past several years, numerous clinical and experimental studies have been performed to investigate the incidence and pathogenesis of bisphosphonate-related osteonecrosis of the jaw (BRONJ). The research has mainly focused on the commonly used potent nitrogen-containing bisphosphonates, which currently play pivotal roles in the treatment of diseases with increased bone resorption, such as osteoporosis, antagonism of hypercalcemia, malignancies and osseous metastasis [1, 2]. Suppression of receptor activator of nuclear factor-κB ligand (RANKL) with subsequent reduced osteoclast activation and bone resorption was described as the key mechanism for the bisphosphonate effects and treatment. To date, many hypotheses have been proposed to explain the pathogenesis of BRONJ. Accumulation of bisphosphonates caused by the high bone turnover within the jaw, along with its toxic effects, is frequently discussed [3, 4].

The common estimation of 190 million prescriptions issued before 2007 and the increased risk from long-term drug exposure may increase the incidence of BRONJ in the

future [5–8]. Even though there is a low cumulative incidence ranging from 0.8 to 12 %, BRONJ will emerge as a severe side pathology in the oncologic and maxillofacial fields [5].

Many efforts have been made to prevent this localized bone pathology, because it is frequently associated with invasive dento-alveolar treatment. Classifications and treatment protocols have been introduced to support the decision-making process and further treatment by clinicians. However, there is still a lack of knowledge concerning the pathogenesis of this bone pathology.

Marx et al. [9] and Ruggiero et al. [10] described an osteopetrotic bone disorder leading to avascular osteonecrosis caused by bisphosphonate treatment. A recent in vitro study sustained this theory by showing significantly higher proliferation of human alveolar cells compared with osteoblasts of the long bone [11]. Furthermore, significant changes in the expression of soft tissue and bone matrix proteins, such as Msx-1, RANKL and bone morphogenetic protein (BMP) 2/4, in the periodontal tissue of BRONJ, leading to positive bone turnover, have been described [12]. Therefore, one can hypothesize that bisphosphonate intake is associated with an increase in the bone mineral density (BMD), i.e., general osteosclerosis of the jaw. BRONJ may be associated with a general increase in the BMD not only in clinically localized bisphosphonate-related osteonecrosis (BRON)-adjacent areas, but also in clinically unapparent areas, suggesting a subclinical, but potential, risk factor. At present, there is a lack of data focusing on BMD determination in various regions of BRONJ and the correlation with the commonly used clinical stages of the American Association of Oral and Maxillofacial Surgeons (AAOMS).

Cone-beam computed tomography (CBCT), which utilizes cone-beam geometry, flat-panel detection and three-dimensional (3D) reconstruction algorithms, has emerged as an important technical advancement in maxillofacial and dental imaging [13, 14], and its usefulness for numerous indications has been proven [15–18]. The relatively low radiation doses with high spatial resolution and accurate 3D views allow thorough information to be obtained for the bone dimensions and quality, e.g., the BMD [19]. Trabecular BMD measurements have been shown to be able to detect slight changes in bone turnover and may potentially be useful in the detection of early stages of bone diseases [20]. Recent advances in CBCT techniques and software tools for post-processing have sparked interest in evaluating this imaging modality as a potentially valuable diagnostic tool for the early detection of BRONJ.

To gain evidence regarding the hypothesized increase in bone mineralization through bisphosphonate treatment, the aims of this study were to compare the BMD measurements obtained by CBCT on various areas of BRONJ and to further correlate these measurements with the commonly accepted stages of the AAOMS.

Patients and methods

Patients

A total of 48 patients were included in this retrospective study, comprising 24 BRONJ patients and 24 age- and sex-matched controls. The 24 BRONJ patients (19 females, 5 males; mean age 70.2 years; age range 46–84 years) had histories of osteoporosis, osseous metastasis and malignancy, and were referred to our Department of Cranio-Maxillofacial Surgery for clinical and radiological evaluation prior to surgery. BRONJ in the patients was characterized and staged according to the BRONJ criteria of the AAOMS. All specimens taken perioperatively showed distinct histopathological aspects of BRONJ. Exclusion criteria for the study were signs of soft and hard tissue inflammation within the jaws, except for regions of exposed bone. Additional exclusion criteria were radiation therapy and disease compromising bone turnover in the patient history. The 24 age- and sex-matched patients (19 females, 5 males; mean age 68.0 years; age range 45–83 years) without soft or hard tissue pathologies, relevant comorbid disease or medical treatment served as a control group. All patients provided informed written consent prior to their inclusion in this study.

CBCT

A KaVo 3-D eXam (KaVo Dental GmbH, Biberach, Germany) with an amorphous silicon flat-panel detector (20 × 25 cm) was used as the CBCT unit. The exposure volume was set at a height of 102 mm, and the voxel size was 0.4 mm. The scan was set at a high-frequency constant potential of 120 kVp, and the occlusal plane of each patient was set parallel to the floor base using ear rods and a chin rest. The patient position was adjusted according to alignment laser beams, and all scans were performed by a uniquely trained team of three experienced dento-maxillofacial radiographers. The calculated digital imaging and communications in medicine data were transferred and evaluated by the system's software viewer (KaVo eXam version 1.8.0.5; KaVo). The CBCT unit and its software were regularly calibrated according to the manufacturer's instructions. The calibration procedure included geometric as well as density measurements with a normalized phantom material. BMD analyses in the regions of interest (ROIs) were performed using algorithms based on the voxel value distribution. The obtained images provided X-ray attenuation information for specific image voxels in

terms of bone density values (BDVs) related to the gray-scale. These data were used to determine the density of the scanned bone.

Measurements

The KaVo eXam version 1.8.0.5 software viewer was used interactively in this study. Each CBCT scan was analyzed by positioning ROIs in the anterior mandible (incisor region), posterior mandible (molar region), anterior maxilla (incisor region) and posterior maxilla (molar region). Three additional ROIs were drawn in the BRON-adjacent regions (anterior, posterior and caudal to the lesion), as indicated in the exemplary distribution shown in a panoramic scout view of the unit in Fig. 1. Measurements were performed exclusively in the multi-planar view. After reorientation of the *x*–*y*–*z* axis according to the axial, coronal and sagittal planes, measurements to determine the BDVs were carried out (Fig. 2). The sizes of the ROIs were standardized between 30 and 40 mm^2 for all measurements of the anterior and posterior regions. The ROI sizes for the BRON-adjacent area measurements were between 20 and 30 mm^2. The authors ensured that only trabecular bone was in the measured fields.

Statistical analysis

Statistical analyses of all data were carried out using SPSS version 18.0 for Mac software (SPSS, Cary, NC, USA). The measurements for the anterior and posterior regions of one half jaw were combined into a single jaw area. Within the BRONJ group, the areas were additionally divided depending on the location of the clinically apparent BRONJ into BRON ipsilateral and contralateral jaw areas. All measurements of the osteonecrotic adjacent ROIs were combined into one BRON-adjacent area.

A comparative analysis of the data was performed for each group and jawbone area. The mean, standard deviation, median, minimum, maximum and confidence interval of the values were calculated for each group. The results were analyzed using Student's *t* test and analysis of variance. A significant difference was assumed if the probability value was less than 0.05.

Ethical statement

The study design met the criteria in paragraphs 4a and b of the guidelines (version 21.5.2010) of the Cantonal Ethics Committee of Zurich, and was therefore exempt from Institutional Review Board approval. The study design thereby fulfilled the guidelines of the Declaration of Helsinki concerning Ethical Principles for Medical Research Involving Human Subjects.

Results

In this study, 24 patients with BRONJ (19 females, 5 males; mean age 70.2 years; age range 46–84 years) and 24 patients without any bone pathology (19 females, 5 males; mean age 68.0 years; age range 45–83 years) were examined. The demographic data (sex, age, disease, type of

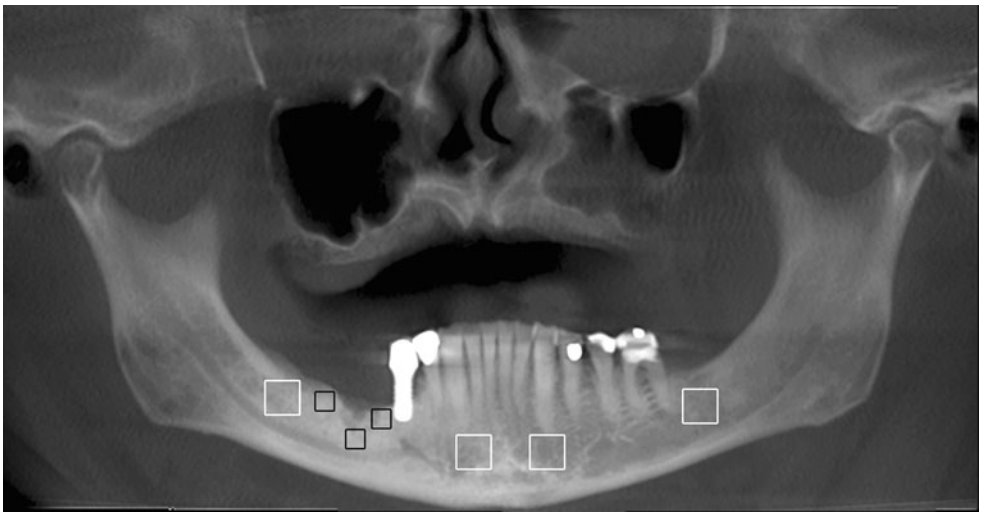

Fig. 1 Panoramic scout view of the CBCT unit showing an exemplary distribution of the ROIs. The anterior and posterior ROIs within the mandible are marked by white squares. The anterior and posterior regions of the right hemi-mandible were combined as one jaw area (BRON ipsilateral area), while the areas of the left hemi-mandible were combined as the BRON contralateral area. The ROIs adjacent to the BRON (anterior, posterior and caudal to the demarcation line) marked by *black squares* were combined into one BRON-adjacent area

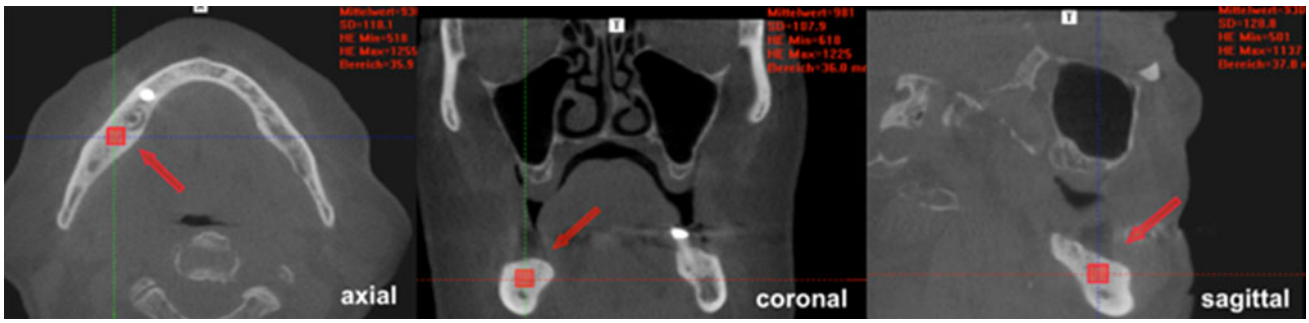

Fig. 2 ROI with the corresponding measured field marked by a *red square* adjusted to the axial, coronal and sagittal planes for 3D BMD evaluation (color figure online)

Table 1 Demographic data for the patients with BRONJ

Sex	Age (years)	Disease	Bisphosphonate	Duration of treatment before BRONJ (months)	History of trauma	Stages[a]
F	68	Breast cancer	Zoledronate	60	Tooth extraction	3
F	75	Thyroid cancer	Zoledronate	36	Tooth extraction	2
F	85	Breast cancer	Zoledronate	48	Tooth extraction	3
F	57	Breast cancer	Zoledronate	48	–	1
F	77	Breast cancer	Zoledronate	8	Tooth extraction	3
F	80	Breast cancer	Zoledronate	9	Tooth extraction	1
F	69	Breast cancer	Zoledronate	22	Explantation	2
F	67	Osteoporosis	Zoledronate		Tooth extraction	1
F	67	Breast cancer	Zoledronate	34	–	2
F	71	Breast cancer	Zoledronate	24	Tooth extraction	2
M	67	Renal cell cancer	Zoledronate		Explantation	1
F	67	Osteoporosis	Alendronate	36	Tooth extraction	2
F	75	Osteoporosis	Alendronate	120	Tooth extraction	2
F	64	Breast cancer	Zoledronate	9	Tooth extraction	2
F	83	Osteoporosis	Ibandronate	12	Poorly fitting denture	2
F	46	Melanoma	Zoledronate		–	2
F	79	Osteoporosis	Ibandronate	18	Tooth extraction	2
F	61	Malignant mesothelioma	Zoledronate	18	Tooth extraction	2
F	75	Osteoporosis	Aledronate	36	–	2
M	70	Multiple myeloma	Zoledronate		Implantation	2
M	65	Multiple myeloma	Zoledronate	36	–	3
F	60	Breast cancer	Zoledronate		Tooth extraction	2
M	79	Prostate cancer	Zoledronate	48	–	1
F	85	Osteoporosis	Pamidronate	36	Tooth extraction	2

[a] According to the stages of the AAOMS

–, No trauma in the history

bisphosphonates, duration of treatment, trauma in history, AAOMS stage) are shown in Table 1.

Overall, the bisphosphonate patients with BRON had significantly higher ($p \leq 0.01$) mean BMDs in the maxilla and mandible within the ipsilateral and contralateral jaw areas compared with the control group (Figs. 3, 4).

Significantly higher BMDs in the BRON-adjacent areas compared with the clinically affected BRON ipsilateral, contralateral and control jaw areas could be measured (Figs. 3, 4, 5, 6). Descriptive data for the evaluated groups and areas are shown in Table 2.

The BMDs of the evaluated areas in BRONJ divided into stage II and stage I according to the AAOMS showed no significant differences ($p \geq 0.05$). No statistical conclusion was reached for stage III disease because of the low number of patients with this stage in the examined group.

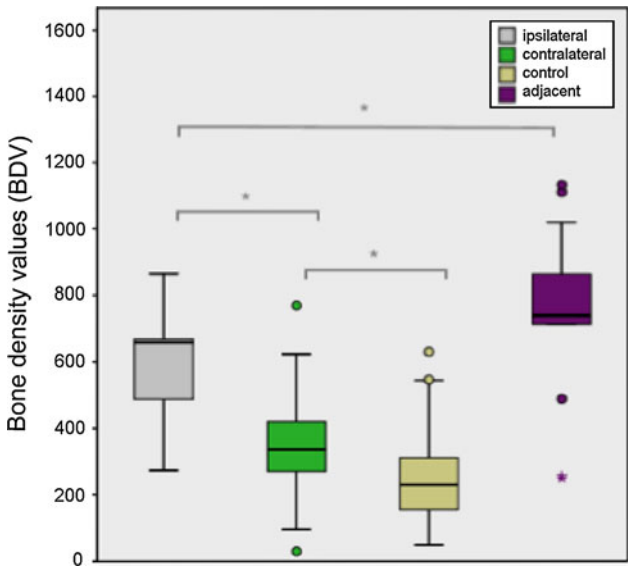

Fig. 3 Box-plot diagram representing the bone density distribution within the maxilla of BRONJ patients (ipsilateral, contralateral and adjacent areas) and the corresponding control group

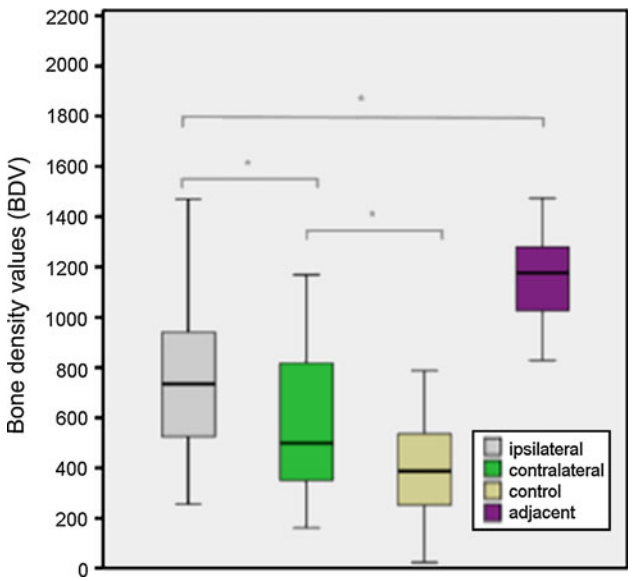

Fig. 4 Box-plot diagram representing the bone density distribution within the mandible of BRONJ patients (ipsilateral, contralateral and adjacent areas) and the corresponding control group

Discussion

During the past decade, many attempts have been made to elucidate the pathogenesis of BRONJ and its incidence in the general population [2, 11, 12].

Recent in vitro studies have indicated that bisphosphonates potently inhibit bone resorption by suppressing the recruitment and activity of osteoclasts, and even by inducing apoptosis, leading to a general decrease in bone resorption [21–23]. Beyond their anti-resorptive properties, nitrogen-bisphosphonates enhance the proliferation of osteoprogenitor cells and osteoblasts, and upregulate BMP-2, collagen type II and osteocalcin, thereby pushing the bone turnover toward increases in bone matrix formation and mineralization [11, 24–26].

In accordance with the current clinical and molecular findings, the aims of this study were to evaluate the BMDs within various jaw areas of BRON patients and to compare these measurements with an age- and sex-matched control group. In the current literature, one can find several radiological studies using CBCT technology for BMD determinations to address various scientific questions [27, 28].

Owing to the low-dose high-spatial-resolution visualization of high-contrast structures and accurate 3D views, CBCT imaging permits quantitative and qualitative evaluations of osseous structures. For these reasons, as well as socioeconomic costs, especially under the premise of prospective regular follow-ups, the authors chose CBCT technology for their BMD evaluations.

The results of the present study revealed significantly higher BDVs in all evaluated areas in patients with BRONJ compared with those in the control group. Significant differences in the BMDs within the BRON ipsilateral jaw areas compared with the contralateral areas were also found. As a consequence, CBCT technology may allow the detection of bisphosphonate-related osseous changes and differences within the jaw.

The areas adjacent to the osteonecrotic sites showed significantly higher BMDs than the ipsilateral or contralateral jaw areas. Exposed bone and delayed wound healing with a nearly obligate involvement of the oral microflora (e.g. *Actinomyces*) will lead to the development of an inflammatory process and further trigger osteosclerotic bone remodeling (condensing osteitis). The latter factors and impaired vascular nutrition may considerably support the hypothesis of avascular osteonecrosis within the jaws [29, 30].

BRONJ may be associated with an increase in BMD not solely in clinically obvious BRON-adjacent areas, but also in clinically unapparent areas, suggesting a subclinical general osteosclerosis of the jaw.

In this study, the authors were unable to detect any significant differences in the BMDs of BRONJ patients between AAOMS stage I and stage II [5]. These findings further underline the heterogeneous clinical picture of this bone pathology. However, coherence between the BMDs and the AAOMS stages is desirable to allow prediction of the genesis of BRONJ. The AAOMS stages represent the course of osteonecrosis progression and complications, but do not consider the general osseous changes within the

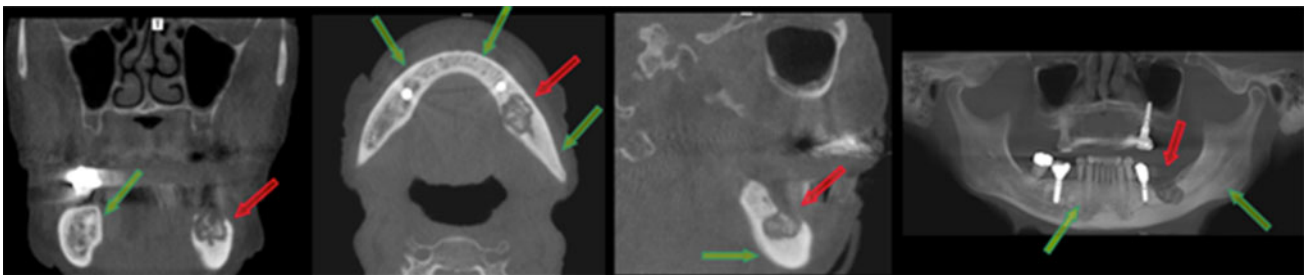

Fig. 5 Exemplary CBCT images of a 70-year-old woman treated with zoledronate for 2 years with a history of metastatic breast cancer. Axial, coronal, sagittal and panoramic scout views showing BRON (*red arrows*) and perspicuous diffuse mandibular osteosclerosis (*green arrows*) are shown (color figure online)

Fig. 6 Exemplary CBCT images of a 75-year-old woman treated with zoledronate for 2.5 years with a history of thyroid cancer and bone metastasis. Axial, coronal, sagittal and panoramic scout views showing marked BRON (*red arrows*) and the corresponding osteosclerosis (*green arrows*) affecting the maxilla are presented (color figure online)

Table 2 Descriptive analyses of the BDVs of the BRONJ (ipsilateral, contralateral and adjacent areas) and control groups

Group	Jaw	Region	Mean	SD	Median	Minimum	Maximum	95 % CI
Bisphosphonate	Maxilla	Ipsilateral	600.93	174.87	659.5	273.67	864.33	475.83–726.03
	Maxilla	Contralateral	360.0	219.48	336.3	29.66	770.0	202.99–517.00
	Maxilla	Control	246.14	122.34	230.16	48.66	630.66	221.87–270.42
	Maxilla	Adjacent	748.13	260.14	740.0	249.3	1132.0	604.07–892.19
	Mandible	Ipsilateral	762.52	299.64	735.50	256.66	1469.66	666.69–858.35
	Mandible	Contralateral	581.42	280.11	500.0	162.33	1169.0	486.64–676.20
	Mandible	Control	388.61	178.32	387.50	25.00	787.66	353.23–424.00
	Mandible	Adjacent	1162.85	165.84	1176.66	828.33	1473.66	1118.84–1206.85

SD Standard deviation, *CI* confidence interval

entire jaw. Therefore, it may be interesting to include BMD measurements obtained by CBCT in the staging of BRONJ, since osteosclerosis may be a potential risk factor for an early stage of the development of this disease. Consequently, clinicians should be aware that the clinical picture of a bisphosphonate-related osteonecrotic lesion may possibly only represent the tip of the iceberg. Hence, further studies are necessary to implement BMD measurements in the early diagnosis of bisphosphonate-related osseous changes.

There are limitations to this study. First, a rather small sample size was investigated. Further studies with larger cohorts including various AAOMS stages as well as an additional group with patients under bisphosphonate treatment without clinically apparent BRONJ are required. Second, CBCT bone density measurements may need to be regarded with caution, since this modality does not yield equally robust bone density measurements compared with established multi-detector CT units. The beam geometry, artifacts (metal implants), imaging parameters and object positioning [31] are known to significantly influence attenuation measurements. Nevertheless, this study design included regular calibration of the CBCT unit. Identical patient positioning, and homogeneous reconstruction algorithms and data acquisition were used in all subjects. Moreover, based on our experience and other studies, a low variance of BDVs occurs in healthy patients [32, 33]. Despite the difficulties in measuring the BDVs, we have shown that the performed technique was sufficient to detect bone pathologies, i.e., bisphosphonate-associated bone

sclerosis versus healthy bone. A systematic evaluation of the stability of BDVs in CBCT and their transferability to other devices lies beyond the scope of this study, but should be addressed in future studies.

Further studies will hopefully be able to establish a radiological classification for BRONJ. This should ideally correspond with the clinical stages and provide clinicians with reliable data about the stage of the disease. For early detection of BRONJ and BRONJ risk evaluation in particular, this classification should be based on CBCT as the 3D imaging technology that is most commonly used in dental surroundings.

Bisphosphonate-related bone pathologies can be detected by CBCT and are associated with increases in the BMD not only in clinically obvious BRONJ, but also in clinically unapparent areas, suggesting a subclinical general osteosclerosis of the jaw. The BDVs obtained by the CBCT device allowed reliable comparisons within this study population owing to the standardized data acquisition. The data transferability to other CBCT devices needs to be further elucidated.

Conflict of interest The authors declare that they have no conflict of interest.

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